ISSN : 2322-0066

Research and Reviews: Research Journal of Biology

Principles Involved in Bioassay by different Methods: A Mini-Review

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Mini-Review

Received: 13/04/2015
Revised: 19/04/2015
Accepted: 05/05/2015

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Keywords: Bioassay, Types of Bioassay, Bioassay for Drugs

ABSTRACT

Bioassay is defined as estimation or determination of concentration or potency of physical, chemical or biological agents by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions. Bioassay is a successful tool in estimation and discovery of biologically active substances and important application in sensitivity and specificity of pharmacological applications. Chemical method is very complex method which requires high chemical dosage and chemical composition shows the pharmacological actions against the drug.

Introduction

Biological assays are methods for the estimation of nature, constitution, or potency of a material by means of the reaction that follows its application to living matter. Bioassay is defined as estimation or determination of concentration or potency of physical, chemical or biological agents by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions [1,2]. An assay is a form of biological experiment; but the interest lies in comparing the potencies of treatments on an agreed scale, instead of in comparing the magnitude of effects of different treatments. Biological assays or biological standardizations or simply bioassays are methods used for estimation of the potency of substances by observing their pharmacological effects on living animals (in vivo) or isolated tissues (in vitro) and comparing the effect of these substances of unknown potency to the effect of a standard.

In this analysis the response produced by the test compound is compared with that of standard sample the way similar to other analytical methods but here the biological system is involved in the determination [3]. Bioassay is Assessment of a biological substance. Bioassay or biological standardization is a type of scientific experiment typically conducted to measure the effects of a substance on a living organism and is essential in the development of new drugs and in monitoring environmental pollutants [4].

Bioassays are based upon the use of biological responses as detection system for biologically active substances. In the simplest form it is used to assay the presence (and concentration) of a particular substance by comparison with a known amount of the same substance. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. Bioassay is a procedure for the determination of the concentration of a particular constitution of a mixture [5-8].

Structure of Biological Assay

The typical bioassay involves a stimulus applied to a subject. Application of stimulus is followed by a change in some measurable characteristic of the subject, the magnitude of the change being dependent upon the dose. The intensity of the stimulus is varied by using the various Doses by the analyst.

Qualitative Assays	Quantitative Assays
These do not present any statisticalanalysis.	These provide numerical assessment of some property of the material to be assayed, and pose statistical problems.

Principle of Bioassay

Active principle to be assayed should show the same measured response in all animal species [9]. Bioassay involves the comparison of the main pharmacological response of the unknown preparation with that of the standard [10-14]. The method selected should be reliable, sensitive, and reproducible and should minimize errors due to biological variation and methodology. The degree of pharmacological response produced should be reproducible under identical conditions. The reference standard and test sample should have same pharmacological effect and mode of action, so that their DRC curve run parallel and their potency ratio can be calculated [15-18]. Activity assayed should be the activity of interest; Individual variations must be minimized/accounted for [19]. Bioassay might measure a diff aspect of the same substance compared to chemical assay. The test solution and standard should be compared for their established pharmacological effect using a specified pharmacological technique [20-23]. Prior to the development of sensitive, precise, accurate chemical and binding displacement assays for the presence or concentrations of drugs or autacoids we had to rely on bio-assays [24-26].

Types of Bioassays

There are three main types of bioassays (other than qualitative assays) [27]

1. Direct Assays
2. Indirect Assays based upon quantitative responses
3. Indirect Assays based upon quantal responses (‘all or none’)

Direct Assay

Doses of the standard and test preparations are sufficient to produce a specified response, and can be directly measured.

Indirect Assay

In indirect bio-assays the relationship between the dose and response of each preparation is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately [29].

Quantal Assay

This response is in the form of ‘all or none’ means no response or maximum response. These can be biossayed by end point method. Predetermined response is measured which is produced by threshold effect.

Quantal Responses are population response based on an all-or-nothing (0 or 1 – presence or absence) response such as death [30-34].

$$\text{Concentration of Unkown} = \frac{\text{Dose of the Standard}}{\text{Dose of the Test}} \times \text{Concentration of Standard}$$

Graded Assay

It is proportional to the dose and response may lie between no response and maximum response [28]. Graded Responses can be any type of measured responses in isolated tissues in particular, but also in whole animals. Such responses are infinitely graded and there are a large number of them. Examples include contractions of muscle, blood pressure, blood sugar concentrations, etc. [35]

Matching Method

In this type of assay the test substance and the standard are applied and the responses obtained are matched by a trial and error process until they produce equal effects [36-38].

This may also limit to analytical dilution assay, as the assay involves the determination of the factor by which the test substance is diluted or concentrated in order to produce response that is equal to that of known amount of the standard drug [39-40]. Its advantage is that it does not depend on the assumption of a dose-response relationship. The main disadvantages are that it is purely subjective, and experimental errors cannot be determined from the assay. It gives no indication or the parallelism of the dose-response curves of the standard drug and test substance, and hence the qualitative differences, as the effects are matched at only one dose level. [41-46]

Advantages: Quick and easy; useful when one is has many samples to test and a semiqualitative answer is sufficient.

Disadvantages: Inherently lacks precision, no accuracy, no D-R data – particularly no data regarding slope. The data is not easily statistically analyzed and probably should not be so analyzed [47].

Bracketing Method

Bracketing bioassay is performed by selecting two standard doses, which will give a close bracket on either side of the response produced by the unknown. The working dose of standard is first determined in the sensitive part of dose-response curve, that is, a dose that will approximately produce 50% of the maximal concentration. The dose of the standard drug is kept constant throughout the experiment, in order to have some idea about the change in the sensitivity of tissue with time. [48-53]

The standard drug is added at fixed intervals but alternating with the test so that each response produced by a dose of test substance is bracketed by responses produced by the dose of standard.

The response of test substance is bracketed between two responses of the standard. Close bracketing gives more accurate results. [54-56]

Interpolation Method

This is a simplest form of graded response assay and involves no statistical data and many calculations. In this assay the dose response curve is fist obtained from different doses of standard ach solution. The concentration of unknown is then read from the standard graph. [57-62]

Interpolation method of bioassay is less time consuming and yet reliable compare to matching type of bioassay. One of the main advantages of this essay is that the sensitivity of the tissue is first determined by prior plotting of a dose response curve with a known agonist as in the case with acetylcholine. If the linearity of curve is good, one can do very accurate estimate of the test substance unknown sample. [63-68]

Three Point Bioassay

In three point bioassay, the DRC of standard & test samples is first obtained from the responses due to graded doses. From the DRC of standard, two standard doses are selected in such a way that they have produced 25% & 50% of the maximal response respectively & are designated as S1 & S2. The responses of these doses lie on the steepest & straightest part (linear) of the curve. From the DRC of test sample one test is selected such that it gives a response which lies in between the two standard responses that is it gives a greater response than S1 & a smaller response than S2 & is designated as T. [69-80]

After selecting the standard & test doses, the bioassay is performed by recording the standard & test responses in randomized fashion as per Latin square design. The pattern of addition of doses is S1, S2, T; S2, T, S1 & T, S1, S2 in 3 successive cycles. The mean values of height of contraction for all the 3 doses are calculated and are used in plotting the graph so as to estimate the potency of the test sample. [81-86]

The precision and reliability of this method is much better than matching and bracketing methods of bioassay & the sensitivity of the isolated tissue preparation is assessed prior to testing the unknown sample. [87-92]

Advantages: Quick and with more precision than a matching assay. There is a possibility of using certain statistical procedures although not with much confidence.

Disadvantages: still an inherent lack of precision, no accuracy [93].

Four Point Bioassay

The classic 2X2 parallel assay involves being able to measure parallelism where drugs acting through the same mechanism are expected to produce parallel dose-response curves [94].

Seed Bioassay

Seeds are living organisms that may be harmed by chemicals. The seed bioassay technology has been implied as decisive and lab scale and method to assess the toxicity of any substance on profitable crops [95-98].

The seedling germination and seedling growth impressions under contrasting concentrations of industrial derivation can give some perception about the abolishing or toxicological impact of industrial effluents on plants. A lab-scale bioassay of distillery effluent was conducted using few profitable cereal crops in order to seed the feasibility of utilizing distillery effluent for crop irrigation purposes [99-102].

Antimicrobial Assay

Standard and clinically isolated microorganism strains were used for antimicrobial assays. A large number of human, animal and plant disease are caused by pathogenic microbes. Infection due to fungi and bacteria has been a major cause of death in higher organisms [103-106]. The discovery of antibiotic penicillin by Fleming is therefore considered to be one of most important discoveries in the world. The microbial assay for antibiotics is a method that uses microorganisms to determine the antimicrobial potency of the antibiotics contained in medicine.

Historically many of the new antibiotics were isolated from natural sources like soil microbes and plants. Many more were later synthesized and introduced in clinical practices [107-112]. Unfortunately human struggle against pathogenic microbes is far from over due to many reasons. Most important of them time to time discovery of new pathogens and remarkable abilities of microbes to develop resistance against used antibiotic. The discovery and development of new antimicrobial agent is therefore an ongoing process. Remarkable diversity of chemicals present in biological samples has tremendous potential in search of new antimicrobial agents [113-119].

Antifungal Assay

Fungal infections have been reported to have dramatically increased in the past decade, and these often occur as systemic infections or as co-infections with other diseases, such as AIDS or cancer, or in patients who are immuno-compromised [120-124]. There is considerable need to discover new fungi-toxic compounds in view of the many plant and human fungal diseases. Some of the common plant fungal diseases are potato late blight, tobacco blue mould, hop downy mildew, Dutch elm disease, ergot of rye, cereal rusts, corn blight and grape downy mildew [125]. The human fungal diseases include athlete's foot, Aspergillosis, Actinomycosis, Histoplasmosis and Corcidiomycosis. The rapid increase in fungal infections and the growing number of new antifungal agents indicate an increasing need for rapid and accurate methods for antifungal screening and susceptibility testing [126-128].

Some fungi can be beneficial to man since they attack harmful insects. The two main methods includes

1. Cylinder Plate Method
2. Paper-disc Method

Antimitotic Assay

Antimitotic agents are defined as any applied stimulus which produces a consistent change/deviation in the mitotic cycle. Inhibition of cell division is a measure of the antimitotic activity of chemical compounds. Antimitotic chemical compounds such as vinblastine and podophyllotoxin have been shown to inhibit cell division of fertilized sea urchin eggs and starfish oocytes. Normally the reaction should be reversible with time, removal of stimulus or on the addition of an antagonist [129-134].

Bioassay for Drugs

Depending upon pharmacological action of various drugs, different preparations may be used. Following chart gives different preparations and the pharmacological activity for which a particular drug is assayed [135-138]. To estimate the emesis activity of digitalis the pigeon preparation is used, guinea

pig preparation is used to assay the cardiac arrest activity. In detail bioassays of some drugs were mentioned in table below (Table 1) [139-148].

Table 1: Preparations for activity assayed on different drugs

Drug	Activity Assayed	Preparation
Histamine	Contractile effect	Isolated, atropinized terminal ileum of Guinea pig.
	Fall in blood pressure	Anaesthetized and atropinized cat.
5 Hydroxy Tryptamine (5HT)	Contractile effect	Isolated atropinized rat uterus, Isolated terminal colon of rat,
	Contractile effect	Isolated fundus strip of rat stomach
	Constriction of blood vessels	Perfused rabbit ear
Acetylcholine	Contractile effect	Isolated rectums abdominus muscle of frog
	Contractile effect	Rat ileum
	Contractile effect	Leech dorsal muscle
	Inhibition of cardiac Contractions	Isolated mouse heart
	Fall in blood pressure	Rat/Cat blood pressure
Noradrenaline	Rise in blood pressure	Blood pressure of the pithed cat
Digitalis	Fall in blood pressure and death	Cat blood pressure
	Stoppage of heart and death.	Guinea pig blood pressure
	Emesis	Pigeon
Adrenaline	Rise in blood pressure	Blood pressure of the spinal cat
	Inhibition of the tone	Isolated rabbit duodenum
	Inhibition of the tone	Isolated caecum of fowl
	Inhibition of the tone	Isolated rat uterus
d-tubo-curarine	Inhibition of the contractile effect	Rat diaphragm with phrenic nerve or Cat gastrocnemius muscle with sciatic nerve.
Insulin	Lowering of blood-sugar Level.	Rabbits
	Convulsions and/or death due to hypoglycemia	Mice
	Increase in glycogen Content.	Isolated rat diaphragm
	Increased metabolism of glucose, indicated by increased in CO2 Production.	Rat's epididymis fat
Heparin	Prolongation of blood clotting time	Whole blood of ox with thrombokinase extract and acetone dried ox brain
Vitamin D	Alleviation of rachitic stage	Rats maintained on richetogenic diet
Antibiotics	Inhibition of growth of micro-organism	Suitable micro-organism grown on suitable nutrient agar medium
Oxytocin	Vasodepressor activity.	Adult cockerel

	Contractile effect	Isolated rat uterus
	Ejection of milk from mammary duct.	Rabbits (female)
Growth Hormone	Gain in weight.	Hypophysectomized rats
	Increase in width of epiphyseal cartilage	
Prolactin	Increase in weight of crop sac.	Cloves of pigeons
Estrogen	Increase in weight of uterus	Rat or mouse (Female)
Progesterone	Proliferative changes in endometrium of uterus.	Sexual immature rabbits
	Increase in carbonic anhydrase-activity in uterus	

Advantages of Bioassays

Sensitivity of the tissue is determined by plotting of a concentrate response curve with unknown agonist. They not only help to determine the concentration but also the potency of the sample. It is especially used to standardize drugs, vaccines, toxins or poisons, disinfectants, antiseptics etc. [149-154] as these are all used over biological system in some or other form. These also help determine the specificity of a compound to be used. The concept and principles of bioassay can be very useful when performing structure activity relationship (SAR) studies as part of the drug invention process when we wish to compare the relative potency and functional effects of different compounds on intact tissue systems [155-169].

Testing of infected patient's sputum helps determine which anti-biotic is given for quick recovery. It is Simple and faster method. Amount of test drug available is small, does not involve complicated calculations and it does not depend on Dose response curve [170-183]. Improvements in chemistry and biochemistry have increased the precision and accuracy of all 'physical' methods for the measurement of the presence and concentrations of drugs, and other pharmacologically active substances, have made many biological-response bio-assays redundant [184-188].

Dose can be plotted even if it varies over thousand fold range. Certain complex compounds like Vitamin B-12 which can't be analyzed by simple assay techniques can be effectively estimated by Bioassays [189-192]. Sometimes the chemical composition of samples is different but has same biological activity and for samples where no other methods of assays are available. Error is normally distributed. [193-198]

Is it useful, therefore, to understand the concepts, principles and techniques of bio-assay as part of the armamentarium of a competent pharmacologist [199-203]. Some researchers fail to recognize the fact that clinical trials have large elements of bioassay, especially when it comes to comparing two drugs for their clinical effects and when given at two or more levels of dosing a classic 'Latin square' design [204-206].

Disadvantages of Bioassay

Sensitivity of the tissue changes with respective time. Dosage timing may vary in mode of application of the drug. It is less accurate, more time consuming and troublesome. Exact match of the response may not be available. Quantitative difference between standard and test may not obtain and Biological variations. [207-219]

Applications of Bioassay

- Establishing regulatory requirements for water quality;
- Ecological monitoring of sewages discharge
- State environmental monitoring of water bodies, particularly in the areas of the human exposure;

- Environmental impact assessment of new technologies, treatment facilities, reconstruction and modernization of national economic projects; for designing local treatment facilities assessment of aquatic ecosystems[220-233].

CONCLUSION

Comparing with chemical assay and physical assay, bioassay is less elaborate, less accurate, more troublesome, more laborious and more expensive. But, it is the only assay to be done if an active principle of drug is unknown and if it is hard to isolate. Chemical method is very complex method which requires high chemical dosage and chemical composition shows the pharmacological actions against the drug. Other purpose of bioassay is to standardize the preparation so that each contains the uniform specified pharmacological activity. In this way, it serves as a pointer in the Commercial Production of drugs when chemical assays are not available or do not suffice. From the clinical point of view, bioassay may help in the diagnosis of various conditions.

It is a successful tool in estimation and discovery of biologically active substances and important application in sensitivity and specificity of pharmacological applications.

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